Effect of Bleeding on the *Modori*-phenomenon and Possible Existence of Some *Modori*-inhibitor(s) in Serum

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(Received December 15, 1989)

Effect of bleeding on the *modori*-phenomenon of gels prepared from rainbow trout muscle in association with death condition and freshness was examined. It was suggested that bleeding might promote the *modori*-phenomenon irrespective of the death condition. Additionally, the *modori*-property changed according to freshness. However, the relationship between the post mortem pH and the *modori*-property was not clearly demonstrated.

Promotion of the *modori*-phenomenon by bleeding suggested the existence of some inhibitor(s) for the *modori*-phenomenon in blood. Actually, the addition of the serum fraction of rainbow trout blood suppressed the *modori*-phenomenon as well as the breakdown of myosin heavy chain.

Recent work in our laboratory have suggested that the *modori*-phenomenon (thermal gel degradation particularly occurs around 60°C) is caused by a group of newly found endogenous serine proteinases having strong degrading action on myosin heavy chain.¹⁻⁴⁾ Additionally, it is also suggested that the *modori*-phenomenon shows remarkable species-specificity.⁵⁾ It was also reported that the *modori*-property depends on various biological factors including death condition,⁶⁾ freshness,⁶⁾ size,⁷⁾ age,⁷⁾ season,^{7,8)} and nutritional condition*² even in the same species.

On the other hand, we found that bleeding may also affect the degree of the *modori*-phenomenon through a series of the study on the *modori*-phenomenon. Therefore, the effect of bleeding in association with death condition and freshness was investigated. The possible existence of some inhibitor(s) for the *modori*-phenomenon in the serum fraction was also described.

Materials and methods

Rainbow Trout

Four rainbow trouts (387-402 g in body weight and 29-30 cm in body length) cultured in the same pond under the same breeding condition in Shiga Prefectural Samegai Trout Farm were used for the experiments. Two were immediately killed by striking in the head after having been taken from

the pond and then one of which was bled as completely as possible from caudal artery by a syringe (11.8 ml of blood was recovered) and the other was not. Another two were transported to the laboratory in Kyoto alive in a polyethylene bag. Afterwards, the fishes were agitated in shallow water for 30 min and then killed by striking in the head. Similar to the immediately killed fish, one was bled (11.5 ml of blood was recovered) and the other was used without bleeding. Because almost the same amounts of blood were recovered from both the immediately and strugglingly killed fishes, the death condition seemed to not affect the bleeding significantly. These four rainbow trouts were stored at 4°C.

Gel Preparation

A small portion (about 20 g) of muscle was excised from each fish at selected time intervals after death. The muscle was minced and ground with 2.5% NaCl for 2 min. Salt-ground meat paste was packed in a small glass tube (diameter, 15 mm; height, 15 mm; thickness, 1 mm) and then heated at 65°C for 2 h. Fairly tough gel was formed once and then gradually degraded during heating. Two hours heating was judged to be appropriate to compare the degree of the *modori*-phenomenon of rainbow trout in the preliminary experiment. It was impossible to make gels from the immediately killed fishes due to the restriction of facilities.

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*² C. N. Wendakoon: Master thesis of Faculty of Agriculture, Kyoto University, Kyoto, 1988, pp. 86-106.

Evaluation of the Degree of the Modori-phenomenon

Sensory evaluation of gel was carried out by an experienced panelist with teeth-cutting and folding tests according to the method of Shimizu *et al.*⁽⁹⁾ The mean score for two test pieces was determined in the teeth-cutting test. The teeth-cutting test was performed by cutting off the test pieces, 15 mm in diameter and 3 mm in thickness, by the fore-teeth and scoring "toughness" according to 10 points scale (10, extremely tough; 8, tough; 6, moderately tough; 4, fragile; 2, very fragile; 0, like mud). The folding test was done by folding the test pieces having the same diameter and thickness as those for the teeth-cutting test neatly in half and evaluating into four grades (A, no crack occurred; B, cracked; C, cracked and separated into two pieces; D, easy to break like mud). The folding test was also examined for about two test pieces.

The breaking strength was measured with Yamaden RE-3305 rheometer equipped with a column type plunger (diameter: 3 mm) at the speed of 1 mm/sec. The average values obtained for two pieces are expressed in the figures and the table.

Measurement of Muscle pH

A small portion of muscle was homogenized with 10 volumes of distilled water and then the pH of the homogenate was measured immediately with a pH meter (Horiba Co. Ltd).

Effect of the Addition of the Serum Fraction Prepared from Rainbow Trout Blood on the Modori-Phenomenon

After coagulating by standing at 4°C for 5 h, collected blood was centrifuged at 2,500 rpm for 10 min. The supernatant was used as the serum fraction. To examine the effect of the serum fraction on the *modori*-phenomenon, the serum fraction prepared from the fish killed while struggling was added to the minced meat of the same fish stored for 24 h prior to the salt-grinding. Bovine serum albumin (Sigma) dissolved in water to give an equal protein concentration to the serum fraction was added to each gel to adjust the added protein concentration same (20.7 mg protein equivalent to bovine serum albumin). The amount of the serum fraction added is expressed as the percentage to the minced meat in Table 1 and Fig. 3.

Estimation of the Breakdown of Myosin Heavy Chain

Gel was homogenized with 10 volumes of dis-

tilled water. One hundred microliter of the homogenate was heated in boiling water for 5 min with 0.1 ml of 100 mM Tris-HCl buffer, pH 6.8, containing 10% SDS and 50 mM 2-mercaptoethanol, and 0.8 ml of 50% glycerol to dissolve the homogenate completely. Thirty microgram protein equivalent to bovine serum albumin was applied on the analysis by sodium dodecyl-sulfate polyacrylamide gel electrophoresis in 10% gel according to the method of Laemmli.⁽¹⁰⁾ Because any polymerized high molecular weight product was not recognized in or on the top of the stacking gel, it seemed no problem to compare each lane in the electrophoretic analysis. Staining was carried out with Coomassie Brilliant blue R-250.

Results

Change in the Modori-Causing Property of Immediately Killed Fish with or without Bleeding

Fig. 1 shows the change of the strength of gels prepared from the immediately killed fishes. Judged from the breaking strength and the sensory score, all gels from the bled fish showed the *modori*-phenomenon (Fig. 1-a), while those from the non-bled fish did not (Fig. 1-b). It is also noteworthy that the evaluation of the gel strength by the breaking strength and the sensory score coincided well.

In addition, it is of particular interest that the degree of the *modori*-phenomenon of the gels prepared from the bled fish changed according to the storage period after death as shown in Fig. 1-a. Gel strength determined by the breaking strength was the weakest at 6 h-storage, and then became stronger 9 h-storage, but was getting weak again 24 h-storage. In this case, the changing pattern of the gel strength was well coincided with that of muscle pH as already suggested by Shimizu and Kaguri.⁽⁹⁾

Change in the Modori-Causing Property of Strugglingly Killed Fish with or without Bleeding

Fig. 2 shows the change in the strength of gels prepared from the fishes killed while struggling. Similar to the case of the immediately killed fish, the *modori*-phenomenon was observed only for gels prepared from the bled fish (Fig. 2-a). It is noteworthy that gels prepared from the fish stored for 6 h became fragile, while fairly tough gels were formed from the fish immediately after death and stored for 3 h. However, different from the case of the fish immediately killed and

Fig. 1. Change in the *modori*-causing property of immediately killed rainbow trout with bleeding (a) or without bleeding (b) during 24 h-storage after death at 4°C. The salt-ground minced meat was heated at 65°C for 2 h. Letters in the figure indicate the grade in the folding-test. Details are described in the text. ●—●, breaking strength; ○—○, score in the teeth-cutting test; △—△, muscle pH.

Fig. 2. Change in the *modori*-causing property of strugglingly killed rainbow trout with bleeding (a) or without bleeding (b) during 24 h storage after death at 4°C. Salt-ground minced meat was heated at 65°C. Letters in the figure indicate the grade in the folding-test. Details are described in the text. ●—●, breaking strength; ○—○, score in the teeth-cutting test; △—△, muscle pH.

Table 1. Effect of the rainbow trout serum fraction on the *modori*-phenomenon*¹

Serum concn. (%) ^{*2}	0	1	2.5	5
Breaking strength (g) ^{*3}	76	103	168	223
Sensory score ^{*3}	2C	3B	4A	6A

*¹ The serum fraction prepared from the strugglingly killed fish was added to the miced meat of the same fish stored for 24h prior the salt-grinding.

*² Amount of the serum fraction added is expressed as the percentage to the minced meat.

*³ Degree of the *modori*-phenomenon was evaluated as described in "Materials and methods".

Fig. 3. SDS-PAGE analysis of the breakdown of myosin heavy chain. The serum fraction obtained from strugglingly killed fish was added to the minced meat of the same fish stored for 24 h. Bovine serum albumin dissolved in water to give an equal protein concentration to the serum fraction was added to each gel to adjust the protein concentration same (20.7 mg protein equivalent to bovine serum albumin). The amount of the serum fraction added is expressed as the percentage to the amount of the minced meat. The arrow indicates the myosin heavy chain band. a, minced meat and 5% serum without heating; b, minced meat and no serum with heating; c, minced meat and 1% serum with heating; d, minced meat and 2.5% serum with heating; e, minced meat and 5% serum with heating.

bled (Fig. 1-a), the decrease of the gel strength did not correspond with the change of muscle pH. The strength of gels prepared from the non-bled fish stored for 6 h became weak, which was not judged as *modori*-gel, presenting no creack in the folding test (Fig. 2-b).

Effect of the Serum Fraction of the Modori-Phenomenon

The results shown in Figs. 1 and 2 suggested the possibility that blood contaminated in non-bled fish meat might suppress the *modori*-phenomenon. To examine this, effect of the serum fraction on the *modori*-phenomenon was investigated. As shown in Table 1, the gel strength was recovered markedly by the addition of 5% of the serum fraction. Moreover, the breakdown of myosin heavy chain, which was supposed to be the cause of the *modori*-phenomenon,¹⁾ was also inhibited

by the addition of the serum fraction (Fig. 3). These results suggest the possibility that the serum fraction contained some inhibitor(s) for the *modori*-phenomenon. The additional possibility that this recovery in the gel strength was due to the gel forming ability of the added serum fraction seems to be eliminated, because the serum fraction itself did not show any gel-forming property at 65°C (data not shown).

Discussion

The results presented in Figs. 1 and 2 suggested that bleeding might promote the *modori*-phenomenon, because the *modori*-phenomenon was observed only for the gels prepared from the bled fish irrespective of the death condition. This was further confirmed in the separate experiments performed on the other day and almost similar results were obtained for other fish species including tilapia and crucian carp (data not shown). Therefore, it was speculated that blood contains some inhibitor(s) for the *modori*-phenomenon. This speculation was confirmed by the experiment shown in Table 1 and Fig. 3. Further purification and characterization of the serum *modori*-inhibitor(s) are now being pursued.

On the other hand, Shimizu and Kaguri⁹⁾ pointed out that the decrease of post-mortem muscle pH might correspond to the enhancement of the *modori*-phenomenon using tilapia. However, relation of post-mortem muscle pH and the degree of *modori*-phenomenon was not clearly demonstrated in our experiment, because the contradictory effect was observed for the gels prepared from the fish immediately killed and bled (Fig. 1-a) and for those from the fish strugglingly killed and bled (Fig. 2-a). In addition, it is also interesting that the increase of pH at 9 h-storage muscle was observed for all fishes. However, no explanation is available for this phenomenon at present.

Except for the case of the immediately killed fish without bleeding (Fig. 1-a), the strength of all gels prepared from the fish stored for 6 h showed the tendency to become weak. The underlying mechanisms, however, still remains poorly understood.

Acknowledgments

The authors are greatly indebted to Dr. Shozo Fushiki and Mr. Toru Kobayashi of Shiga Prefectural Samegai Trout Farm for their kind gift

of rainbow trouts. This study was partly supported by a grant from Towa Food Research Foundation.

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